International Acceptance of the Nonradioactive LLNA: BrdU-ELISA for Evaluating Allergic Contact Dermatitis Hazards

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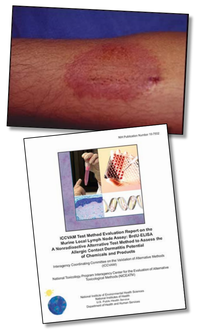
Introduction

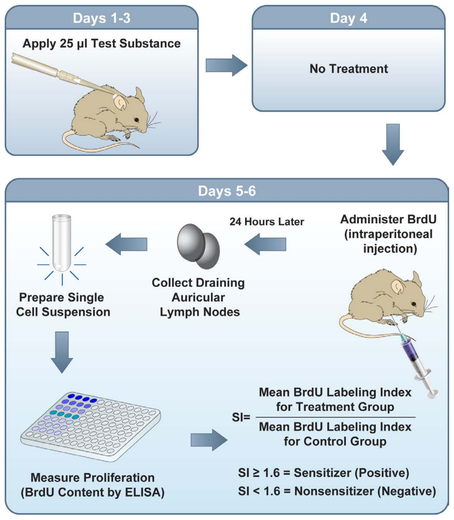
- The murine local lymph node assay (LLNA) is a test method for assessing the potential of substances to cause allergic contact dermatitis (ACD). ACD is an allergic skin reaction characterized by redness, swelling, and itching that can result from repeated contact with a sensitizing substance.
- In response to a nomination by the U.S. Consumer Product Safety Commission in 2007, NICEATM evaluated the nonradioactive LLNA: BrdU-ELISA (**Figure 1**) to assess the ACD hazard potential of substances.
- Takeyoshi et al. developed the LLNA: BrdU-ELISA (Takeyoshi et al. 2001).
  - Measures BrdU incorporation in draining auricular lymph nodes as a measure of lymph node cell proliferation
- ICCVAM published recommendations on the LLNA BrdU-ELISA in a test method evaluation report (available on the NICEATM–ICCVAM Web site at: http://iccvam.niehs.nih.gov/methods/immunotox/llna-ELISA/TMER.htm).



LLNA: BrdU-ELISA Test Method Protocol

- The LLNA: BrdU-ELISA protocol (**Figure 1**) is the same as the traditional LLNA protocol except:
  - It measures BrdU incorporation into lymph node cells via ELISA as a measure of proliferation (instead of ^{3}H-thymidine via scintillation counter in the traditional LLNA)
  - BrdU is injected intraperitoneally instead of intravenously through the tail vein
- The reduced LLNA: BrdU-ELISA (rLLNA: BrdU-ELISA) should be considered and used to determine the ACD hazard potential of chemicals and products in testing situations where dose-response information is not required, or negative results are anticipated.
  - Like the reduced LLNA (ESAC 2007; ICCVAM 2009; Kimber et al. 2006), the rLLNA: BrdU-ELISA protocol uses only the high dose and thereby reduces animal use by up to 40%.
  - If existing information suggests a substance might have ACD hazard potential *and* dose-response information is needed, consider testing in the multidose LLNA: BrdU-ELISA.

Figure 1. LLNA: BrdU-ELISA Test Method Protocol



Abbreviations: SI = stimulation index.

Current Validation Status of the LLNA: BrdU-ELISA

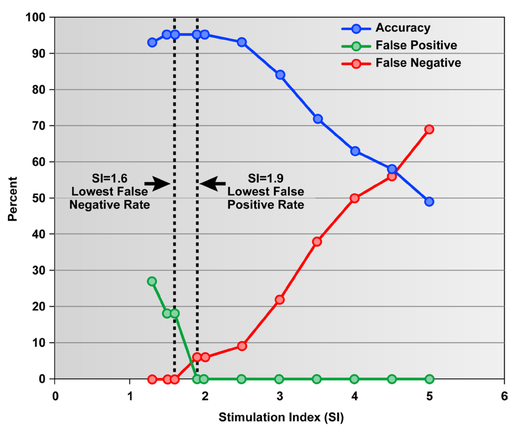
Accuracy

- Accuracy was assessed using a LLNA: BrdU-ELISA database of 43 substances.
  - Kojima et al. 2008 (interlaboratory validation study)
  - Takeyoshi et al. 2003; 2004a and b; 2005; 2006; 2007, and unpublished data
- LLNA: BrdU-ELISA results were compared to traditional LLNA data.
- Stimulation index (SI) ≥ 1.6 produced optimal results based on no false negatives (**Figure 2**).
- LLNA: BrdU-ELISA correctly identified all 32 LLNA sensitizers and 9/11 LLNA nonsensitizers.
  - Accuracy = 95% (41/43)
  - False positive rate = 18% (2/11)
    - Hexane and lactic acid: 1.6 < SI < 1.9
  - False negative rate = 0% (0/32)

Reliability

- Determined extent of agreement of LLNA: BrdU-ELISA outcomes for 18 substances (13 LLNA sensitizers and 5 LLNA nonsensitizers) with multiple test results (intra- and interlaboratory comparisons included).
  - Complete agreement for 85% (11/13) of the sensitizer outcomes.
    - Two substances (hydroxycitronellal and linalool) produced SI < 1.6 in one test and SI > 1.6 in another test.
  - Complete agreement for 80% (4/5) of the nonsensitizer outcomes.
    - Two substances with concordant results were false positive in LLNA: BrdU-ELISA: hexane (2/2 tests had SI ≥ 1.6) and lactic acid (3/3 tests had SI ≥ 1.6).
    - 71% (5/7) agreement for the discordant nonsensitizer (isopropanol).

Figure 2. SI Decision Criteria Performance of the LLNA: BrdU-ELISA Compared With the Traditional LLNA Using 43 Substances



Compared to traditional LLNA results, the lines show the change in performance characteristics for the LLNA: BrdU-ELISA with the SI used to identify skin sensitizers. This analysis used LLNA results for 32 sensitizers and 11 nonsensitizers. For 18 substances with multiple LLNA: BrdU-ELISA test results, the most prevalent outcome was used.

LLNA: BrdU-ELISA Test Method Usefulness and Limitations

- The LLNA: BrdU-ELISA can be used to identify potential skin sensitizers or nonsensitizers.
  - Use SI ≥ 1.6 to identify potential skin sensitizers.
- A slight potential for false positives with borderline weak positive responses (1.6 < SI < 1.9) exists.
  - Consider additional information such as dose-response relationship strength, statistical significance, evidence of systemic toxicity, and/or excessive skin irritation together with SI values.
- The LLNA: BrdU-ELISA might not be appropriate for testing classes of materials with properties that interfere with the assay.
  - Unlike the traditional LLNA, the LLNA: BrdU-ELISA can be used to test nickel compounds, based on its ability to correctly identify these compounds as potential skin sensitizers.

LLNA Peer Review Panel Meetings

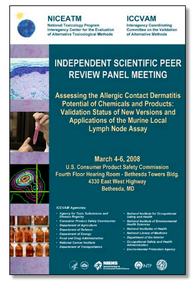
- Public meetings of an international independent scientific peer review panel were held at the Consumer Product Safety Commission in Bethesda, MD, on March 4-6, 2008, and at the National Institutes of Health in Bethesda, MD, on April 28-29, 2009.

Charge to the Peer Review Panel

- Review the draft Background Review Document (BRD) for errors and omissions
- Provide conclusions and recommendations on the current validation status of the LLNA: BrdU-ELISA
- Does the information contained in the draft BRD support ICCVAM's draft test method recommendations?

Peer Review Panel Conclusions

- Concurred that the available data and test method performance supported the use of the LLNA: BrdU-ELISA to identify substances as sensitizers and nonsensitizers, with certain limitations
- Recommended that before animal testing is conducted, consideration be given to the necessity for the substance to be tested for skin sensitization potential
- The complete LLNA Peer Review Panel Reports can be accessed at:
  - http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2008.pdf
  - http://iccvam.niehs.nih.gov/docs/immunotox_docs/LLNAPRPRept2009.pdf



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International Acceptance of the LLNA: BrdU-ELISA

- ICCVAM agreed with the OECD Expert Consultation Group that a single SI ≥ 1.6 to classify substances as skin sensitizers would avoid false negative and indeterminate results, which are not useful for regulatory purposes.
- OECD Test Guideline 442B Skin Sensitization: Local Lymph Node Assay: BrdU-ELISA, which includes the SI ≥ 1.6 to classify substances as skin sensitizers, was adopted on July 22, 2010 (OECD 2010).
- OECD Test Guideline 442B can be accessed at: http://www.oecd-ilibrary.org/
- International acceptance of the LLNA: BrdU-ELISA is expected to result in broader use of LLNA tests.
  - Will further reduce and refine animal use for ACD hazard assessments on a global basis, while ensuring human safety
  - Will reduce costs and environmental hazards associated with the use of radioactive substances

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References

ESAC. 2007. ESAC statement on the reduced local lymph node assay. ECVAM, Joint Research Centre, Institute for Health and Consumer Protection.

ICCVAM. 2009. ICCVAM Test Method Evaluation Report. The Reduced Murine Local Lymph Node Assay: An Alternative Test Method Using Fewer Animals to Assess the Allergic Contact Dermatitis Potential of Chemicals and Products. Available at: http://iccvam.niehs.nih.gov/methods/immunotox/LLNA-LD/TMER.htm.

Kimber I, et al. 2006. Contact Dermatitis 54:181-185.

Kojima H, et al. 2008. Inter-laboratory Validation Study on LLNA-BrdU [Abstract]. 47th Annual Meeting of the Society of Toxicology.

OECD. 2010. Test No. 442B. Skin Sensitization: Local Lymph Node Assay: BrdU–ELISA. In: OECD Guidelines for the Testing of Chemicals, Section 4: Health Effects. Available at: http://www.oecd-ilibrary.org/environment/test-no-442b-skin-sensitization_9789264090996-en.

Takeyoshi M, et al. 2001. Toxicol Lett 119:203-208.

Takeyoshi M, et al. 2003. Toxicology 191:259-263.

Takeyoshi M, et al. 2004a. J Appl Toxicol 24:77-81.

Takeyoshi M, et al. 2004b. Exp Anim 53:171-173.

Takeyoshi M, et al. 2005. J Appl Toxicol 25:129-134.

Takeyoshi M, et al. 2006. J Appl Toxicol 26:5-9.

Takeyoshi M. 2007. Promising Endpoint and Assay Performance of Non-radioisotopic Local Lymph Node Assay Based on BrdU Incorporation [Abstract]. 6th World Congress on Alternatives & Animal Use in the Life Sciences.

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NIEHS National Institute of Environmental Health Sciences

NTP National Toxicology Program
U.S. Department of Health and Human Services